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16. ABSTRACT

On Day 5 of postnatal life, rat pups received a single injection of triethyltin and were later tested for a variety of developmental and adult behaviors. A within-litter dosing design was used with one male and one female from each litter (N=8 pups/litter) receiving either 0 (normal saline vehicle), 3, 6 or a high dose of either 9 or 12 mg/kg triethyltin bromide (TET). The high doses of TET produced 50% and 80% mortality, respectively. For the 3 and 6 mg/kg groups, TET-exposure resulted in a transient decrease in body weight, and a permanent decrease in brain weight. Preweaning TET-exposed pups were less successful in descending a rope, and were less active in both a homing orientation test and a figure-eight maze. When tested as adults, however, these animals were consistently more active than controls in the figure-eight maze. These results indicate that a single exposure to TET in the developing rat, unlike the adult, produces permanent alterations in both brain and behavior. Acute postnatal exposure to toxicants may have general applicability as a model for developmental neurotoxicity.

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Developmental and Behavioral Effects of Early Postnatal Exposure to Triethyltin in Rats

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Triethyltin Postnatal exposure Behavior Development Rats

THE development of the mammalian nervous system occurs over a protracted period of time, beginning early in gestation and extending into the postnatal period. Different components of development, including cell division, migration, and differentiation are vulnerable to disruption by a variety of factors, including chemical exposure [32]. In order to assess the consequences of toxicant exposure on the developing nervous system, several exposure models have been employed [36] which include exposure during some portion of the prenatal or early postnatal period. Considering the rapid rate of neural development it is predictable that the functional consequences of exposure will differ with the period of exposure. In fact, this approach has been successfully employed to define the normal timing and sequence of functional development associated with various brain structures [12, 13, 25].

In the rat, the processes of neural cell division and maturation are still active postnatally [1]. Dobbing [8] reported that the rat brain undergoes rapid growth, termed the brain "growth spurt", during the early postnatal period. Although most postnatal cell division is associated with glia, postnatal neurogenesis has been demonstrated in the hippocampus, cerebellum, and olfactory bulbs [1]. Furthermore, quantitative histological studies indicate that few dendrites are present in the rat prenatally, whereas adult levels are reached by postnatal Day 12 [9]. Early postnatal exposure to toxicants in the rat should, therefore, evaluate the potential for a chemical to disrupt neural development.

Certain toxicants which produce long-lasting behavioral deficits when administered to the developing rat also produce hypoxia. Carbon monoxide produces hypoxia by interfering with the oxygen carrying capacity of hemoglobin. Culver and Norton [7] demonstrated that acute exposure to carbon monoxide in 5-day-old rat pups produced hyperactivity which was present between 4–6 weeks of age. Lead disrupts a variety of mitochondrial functions including uncoupling oxidative phosphorylation and inhibiting respiration [3], and therefore high levels of exposure would produce cytotoxic hypoxia. Several investigators have demonstrated that early postnatal exposure to *high* levels of inorganic lead also produces hyperactivity in the rat [20, 21, 26]. The present study was designed to test the hypothesis that a toxicant which disrupts energy metabolism during early postnatal development will produce neurotoxicity characterized by hyperactivity. Because of its known toxicity, triethyltin (TET) was chosen as a prototype compound to test this hypothesis.

TET inhibits both oxidative phosphorylation and coupled respiration in isolated mitochondria [29] and inhibits glucose oxidation both *in vivo* [5] and *in vitro* [16]. Exposure of adult animals results in splitting of the myelin sheath at the intraperiod line which dilates and fills with fluid [17]; total myelin content of the brain is also reduced [10]. In the neonate, the degree of cerebral edema produced by TET exposure is less than in the adult [30,31]. Since myelin formation begins between 7–10 days postnatally in the rat [14], TET administration at an early age would have only limited potential for producing myelinopathy (vacuolation), although myelin development could be impaired.

In the adult rat, TET produces behavioral changes which

are reversible following termination of exposure [24,28]. However, the behavioral toxicity of TET in the developing rat had not been reported. In the present experiment, therefore, TET was administered to rat pups on postnatal Day 5 and pups were tested for physical maturation, development of reflexes, motor coordination and motor activity. These animals were also tested for motor activity as adults to determine if behavioral impairment was reversible. A preliminary report of these findings has been published elsewhere [23], and since then, the developmental toxicity of TET has also been reported in a study using a similar postnatal exposure [11].

EXPERIMENT 1

METHOD

Animals

Pregnant CD rats (Charles River;Wilmington, MA) were obtained 2 days after mating and housed singly in cages measuring 45×24×20 cm. On the day of parturition (Day 0), pups were randomized and each dam was assigned 4 male and 4 female pups. At 21 days of age, animals were weaned and littermates were housed by sex in groups of 3 or 4 in the same size cages. Animals were maintained on a 12:12 hr light:dark cycle beginning at 0600 hr, in a temperature (22°C±2) and humidity (50%±10) controlled animal facility. Food (Purina Lab Chow) and water were available ad lib throughout the experiment. All behavioral testing was conducted during the diurnal period.

Dosing

On Day 5, each pup received a single intraperitoneal injection of either triethyltin bromide as the salt (TET, Alfa Products; Danvers, MA) or vehicle (sterile isotonic saline) in a volume of 10 μl/gm of body weight. Two groups of animals, 10 litters/group, were used in this study. Group I pups were injected with either 0, 3, 6, or 12 mg/kg TET. A within-litter design was used for dosing: one female and one male from each litter received each dose and therefore each litter contained all treatments. Group I animals were subjects for all tests except the development of open field activity. For the developmental open field testing, a separate group of animals, Group II, was subsequently used. Because all but 2 of the animals receiving 12 mg/kg TET(Group I) died within 3–8 days of dosing, pups in Group II received either 0, 3, 6 or 9 mg/kg TET. Nevertheless, by Day 14, half of the 9 mg/kg TET pups had died, and testing was, therefore, discontinued for this group.

Body Weight

Rats were weighed at 5 days of age and every 3 days thereafter until 20 days of age. After weaning, animals were weighed approximately once a month throughout testing. At 1 year of age, animals were sacrificed and wet weights of whole brains were recorded.

Developmental Testing

Several developmental tests were adapted from those reported by Altman and Sudarshan [2]. Diagrams of the apparatus, further descriptions of the tests, and developmental trends of normal animals may be found in that article. For this study, the age at testing and the criteria for each task (A–G) are shown in Table 1.

A. Negative geotropism: Each pup was placed on a 17° inclined surface with its head pointed downward, and was allowed 180 sec to turn 180°. If the pup did not complete the turn, it was assigned the maximum latency of 180 sec.

B. Placing reaction (chin elicited): Each pup was held by the nape of the neck and touched lightly on its chin with a rod. No response to the rod was scored as 0; slight lifting of the forelimbs without contact with the rod as 1; slow and uncertain lifting of the forelimbs with extending paws and spreading digits was rated 2; swift raising of the shoulders and forelimbs with hindlimbs displaying treading movements was scored 3.

C. Elevation of head: Each pup was observed for 1 min in an empty clear plastic cage. The amount of time in which the head was clearly raised above the surface of the cage was recorded.

D. Ascending on a wire mesh surface: A 45×15 cm wooden frame, covered with 6 mm wire mesh was constructed at a 70° angle with a platform at the top. This apparatus was placed with the bottom resting in cold water (approximately 15°C). Three littermates were placed on the platform and individual pups were then removed and placed on the wire mesh with their hindlimbs touching the water. Latency to ascend to the platform was recorded, with a maximum set at 180 sec.

E. Acoustic startle response: Each pup was held by the nape of the neck, and a toy clicker was sounded immediately behind the pup's head. Three daily trials were given until a criterion was reached of a muscle jerk in any of the extremities on at least 1 trial on 2 consecutive days.

F. Eye opening: Pups were observed for complete opening of both eyes.

G. Air righting reflex: Each pup was held in a supine position and dropped from a height of 20 cm onto a bed of wood shavings. Pups were tested until the ability to right in mid-air and land on all four feet was demonstrated on 2 out of 3 daily trials for 2 consecutive days.

H. Descending on a rope: From 15–20 days of age, pups were tested daily for the ability to descend a 30 cm×17 mm rope suspended above the home cage. Each pup was held by the nape of the neck and placed against the rope with its head pointing upward. Pups were allowed 180 sec to descend without falling.

I. Open field: Pups were observed for 5 min at 10, 15, 20 and 25 days of age in an open field measuring 90×90×60 cm and divided into sixteen 22.5 cm squares. The pup was placed in the center of the field and a crossover was counted each time a square was entered with all four feet.

Postweaning Testing

A. Activity in a figure-eight maze: Locomotor activity was measured at various ages in a figure-eight maze [22]. The maze is a series of interconnected alleys (10×10 cm) converging on a central arena and covered with transparent acrylic plastic. Motor activity is detected by 8 phototransistor/photodiode pairs. Rats were tested individually for 1 hr at 21, 57, 112–118 and 238 days of age, and for 23 hr at 258 days of age.

B. Open field: At 110 days of age, behavior was measured for 5 min in the open field described above. Activity was measured as latency (time from the beginning of the test to the first crossover), crossovers (counted each time an animal entered a square with all four feet), central tendency (crossovers within the 4 central squares) and rears (counted each

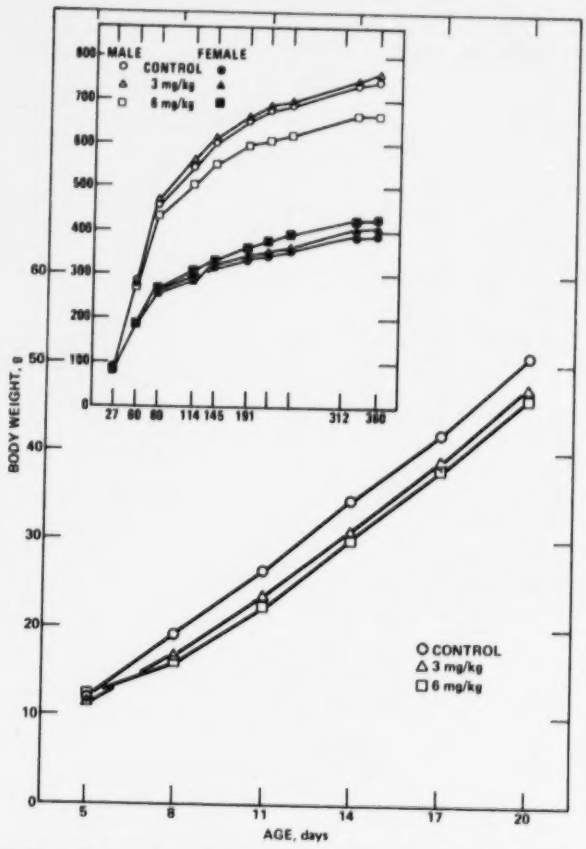

FIG. 1. Body weights of control and TET-dosed rats. Values are expressed as group means (N=8–10/group). Values for the standard error of the mean for preweaning body weights do not exceed the size of the symbols. Acute exposure to TET on postnatal Day 5 produced a slight growth depression during the preweaning period but not during the postweaning period.

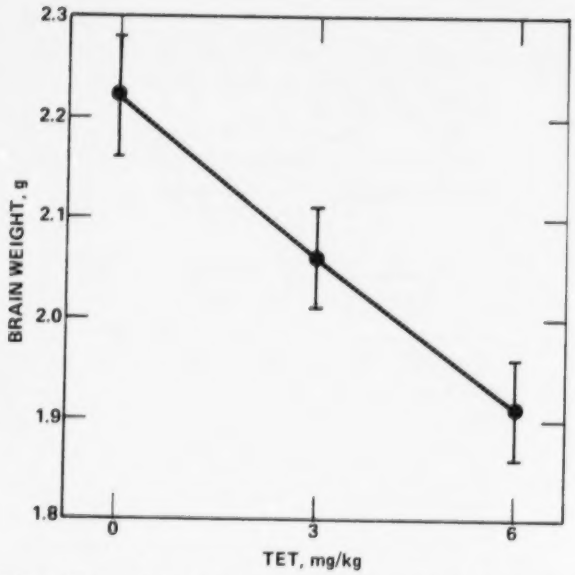

FIG. 2. Brain weights in one-year old control and TET-dosed rats. Data are pooled for males and females and are presented as means ± SE (N=18–20/group). TET exposure produced a dose-related decrease in brain weight.

time an animal raised both front paws off the floor). Testing was conducted under normal lighting, and activity was monitored on closed circuit television by observers in an adjacent room.

Statistical Analysis

For body weight and behavioral tests with repeated measurements, data were analyzed by a multivariate general linear model [19]; when significant differences were found, individual data points were analyzed by ANOVA. For tests with a single measurement, rope descent (scores collapsed across days), adult open field, 23 hr maze activity and brain weight, individual ANOVAs were performed. Values greater than the critical value at $p<0.05$ were accepted as significant. *Post hoc* comparisons were made using Tukey's (a) test [34].

RESULTS

Body weights for Group 1 animals are shown separately for preweaning and postweaning weights in Fig. 1. Preweaning TET-dosed rats weighed significantly less than controls, $F(12,96)=3.49$, $p<0.0003$. At postweaning ages, males weighed more than females, $F(10,44)=55.71$, $p<0.0001$, but there were no differences due to treatment. Thus, TET exposure resulted in a transient decrement in growth during the preweaning period.

Brain weight (Fig. 2) at 12 months of age was significantly decreased by TET, $F(2,50)=18.23$, $p<0.0001$. No independent sex effect or sex by treatment interaction was found, so brain weights for males and females were combined in Fig. 2. Although there was no significant treatment effect on body weight at this time, there was a direct relationship between brain weight and body weight i.e., body weight was a significant covariate of brain weight, $F(1,50)=25.61$, $p<0.0001$. The 7.2% decrease in brain weight at 3 mg/kg, and the 14% decrease at 6 mg/kg were both significantly different from control brain weights ($p<0.01$).

Developmental Testing

Results of developmental testing are shown in Table 1. For negative geotropism, on each of the 3 days of testing, 10–25% of the control pups and 5–15% of the TET-dosed pups fell or rolled off the incline; this was an unexpected finding. However, there were no statistically significant differences between treatment groups, $F(6,102)=0.882$, $p<0.512$. There were also no group differences in the placing reaction or in the time spent with the head elevated. For the task of ascending a wire mesh surface, most pups fell on the first day, but all became more proficient with repeated testing; there was no treatment effect. The age of development of the acoustic startle, eye opening and air righting also showed no indications of treatment effects.

TABLE 1

DEVELOPMENTAL MEASUREMENTS IN CONTROL AND TET-DOSED RATS
(N=17–20/DOSE)

Task Description	Measurement	Age (Days) at Testing	Dose of TET (mg/kg) 0	3	6
Negative Geotropism	Latency to turn 180° (sec)	6	108 ± 15	129 ± 13	142 ± 10
		7	68 ± 14	50 ± 11	64 ± 13
		8	58 ± 15	46 ± 12	54 ± 13
Placing Reaction (Chin Elicited)	Strength of Response (0–3)	9	1.0 ± 0.3	1.6 ± 0.6	1.3 ± 0.3
Elevation of Head	Time (sec) spent with head elevated in 1 min	9	0.8 ± 0.4	4.6 ± 1.7	3.4 ± 1.5
		12	4.8 ± 0.1	5.3 ± 1.1	4.3 ± 0.8
Ascending on a wire mesh	Latency to ascend to top (sec)	11	162 ± 10	174 ± 6	180 ± 10
		12	114 ± 16	123 ± 5	155 ± 11
		13	121 ± 15	119 ± 14	98 ±14
		14	88 ± 15	76 ± 13	52 ± 3
Acoustic Startle	Age (days) at development	10–14	13.0 ± 0.2	13.4 ± 0.2	13.4 ± 0.1
Eye Opening	Age (days) at development	—	15.4 ± 0.2	15.4 ± 0.2	15.4 ± 0.1
Air Righting Reflex	Age (days) at development	16–19	18.2 ± 0.2	18.4 ± 0.2	18.6 ± 0.1

Data on descending a rope are shown in Fig. 3. Pups were scored as either successful (descended without falling) or unsuccessful (fell). The data were combined for all test days and a proportion was obtained. An arcsin transformation was performed on these proportions and an ANOVA was conducted. This analysis indicated a significant effect of treatment, $F(2,45)=9.84$, $p<0.0003$. The 6 mg/kg TET-dosed rats were less successful than either controls ($p<0.01$) or 3 mg/kg TET rats ($p<0.05$) in completing this task.

Ambulation increased with age in the open field as shown in Fig. 4, but there was no significant treatment effect on number of crossovers.

Postweaning Testing

Locomotor activity in the figure-eight maze is shown in Fig. 5. Data analysis of $\log_{10}$ scores showed an overall treatment effect, $F(2,53)=9.09$, $p<0.0005$. The 6 mg/kg TET-dosed rats were more active than both control rats ($p<0.0003$) and 3 mg/kg TET rats ($p<0.002$). Also females were more active than males, $F(1,53)=24.93$, $p<0.0005$), but there was no interaction of treatment with sex, $F(2,53)=1.19$, $p<0.314$.

Hyperactivity persists over a 23 hr period as shown in Fig. 6. Analysis of $\log_{10}$ transformed total 23-hr counts (shown in the insert) indicated that 6 mg/kg TET-dosed rats were hyperactive, $F(2,52)=7.38$, $p<0.01$, in comparison to both control ($p<0.05$), and 3 mg/kg TET-dosed rats ($p<0.05$). Again, females were more active than males, $F(2,53)=15.96$, $p<0.01$.

Measurements of activity in the open field are summarized in Table 2. Activity counts in the figure-eight maze for the first 5 min of a 1-hr test at 110 days of age are also included. There was no treatment effect on activity measured for 5-min in either the open field or the figure-eight maze. However, females were more active than males, $F(5,49)=5.69$, $p<0.0004$. Univariate analysis showed the sex

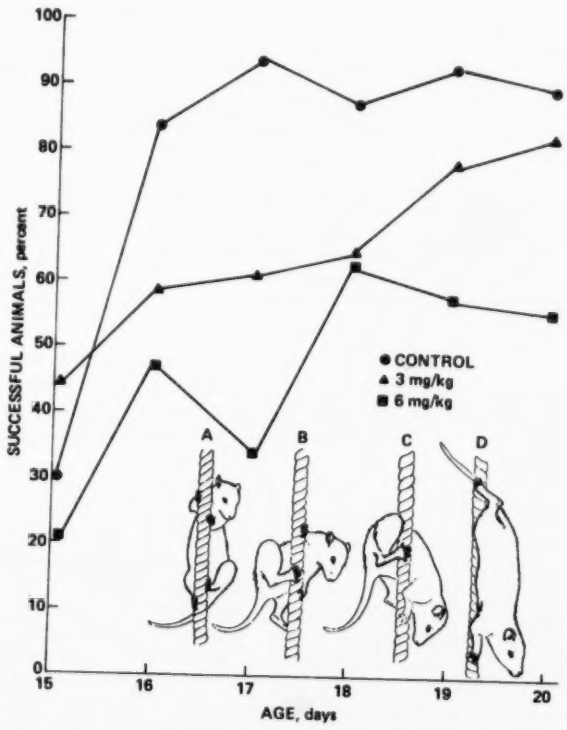

FIG. 3. Rope descending in control and TET-dosed rats. Data are presented as the percentage of animals successfully descending the rope at various ages (N=16–20/group). The mature response pattern is shown in the insert (redrawn from Altman and Sudarshan, 1975). TET exposure produced a significant decrement in the development of this response.

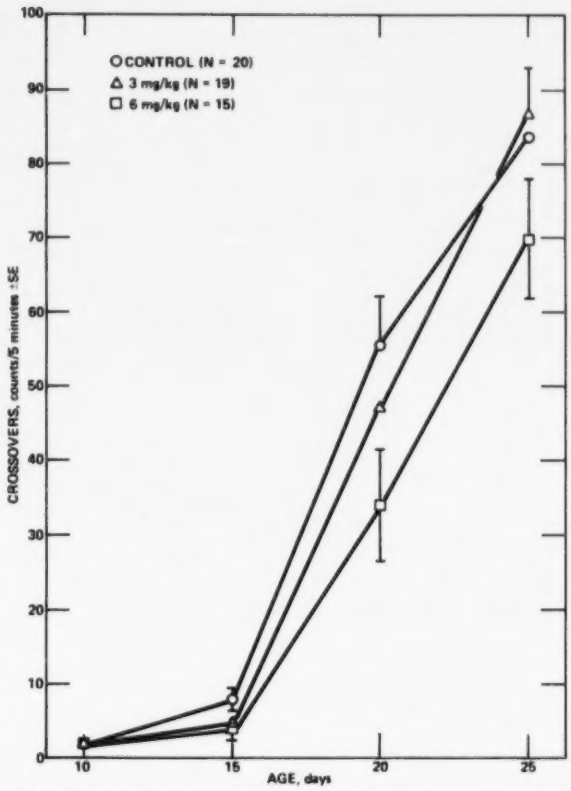

FIG. 4. Development of open field activity in control and TET-dosed rats. There was a progressive increase in the number of crossovers recorded during a 5-min test period between 10 and 25 days of age. Data are combined for males and females (N=15–20/group). TET exposure did not significantly alter the development of open field activity.

difference in crossovers, $F(1,53)=4.58$, $p<0.035$; rears, $F(1,53)=5.50$, $p<0.022$; and 5-min maze activity, $F(1,53)=5.57$, $p<0.02$.

EXPERIMENT 2

METHOD

In a second study, the effects of TET exposure on the development of motor activity were further examined. To simplify testing, each litter was reduced to 4 males at parturition and injected on Day 5 with either 0 or 6 mg/kg TET. A total of 10 control and 12 TET-dosed litters were tested. From 2 to 10 days of age, homing orientation was measured in 4 control and 4 TET-exposed litters; on Day 14, all 22 litters were reduced to 3 pups and the development of 23-hr activity in the figure-eight maze was measured on Days 15 through 21.

Developmental Testing

Homing orientation: Rats were tested for development of nonambulatory homing orientation [2]. This behavior was measured in a circular arena (11 cm in diameter), as shown in Fig. 7, which was positioned between two alleys (7.5 cm long) leading to either the home cage containing mother and littermates or to a cage containing only clean bedding. Pups were tested daily for 180 sec and their orientation was scored at 10 sec intervals. Rats were initially placed in the arena at a right angle to both alleys. Direct orientation towards the home cage or the empty cage was scored as +1 or −1, respectively; intermediate orientations were scored as $+1/2$ or $-1/2$.

Since the development of motor activity confounded orientation in this study, the 10 sec scores were also used to estimate the development of ambulation. A crossover was

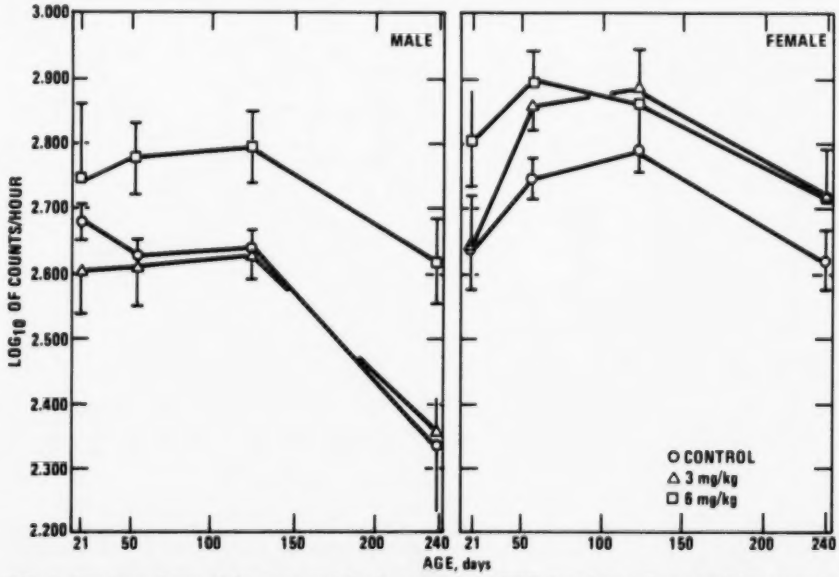

FIG. 5. One hour figure-eight maze activity in control and TET-dosed rats. Log_{10} activity scores are presented separately for males and females (N=8–10/group) at various ages. Brackets represent ± SE. Acute exposure to 6 mg/kg TET on postnatal Day 5 resulted in a persistent hyperactivity.

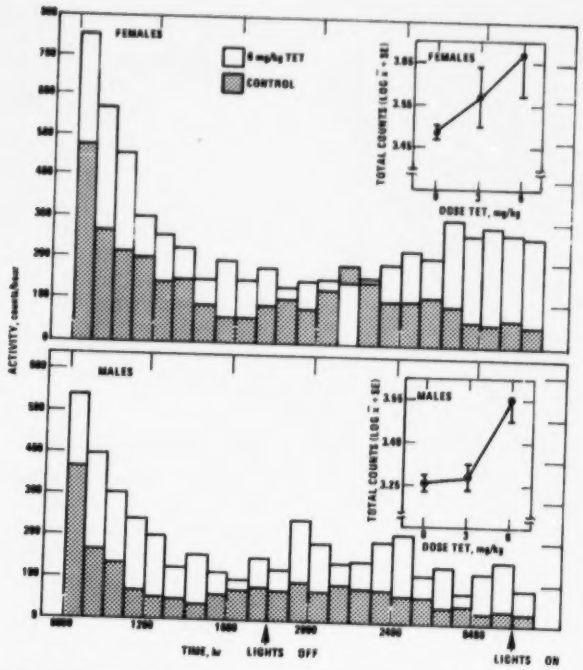

FIG. 6. Figure-eight maze activity of control and 6 mg/kg TET-dosed rats. Individual rats (258 days of age) were tested for 23 hr; values are expressed as mean activity for 1 hr intervals for males and females (N=9–10/group). Group means for the total 23 hr activity are shown in the insert.

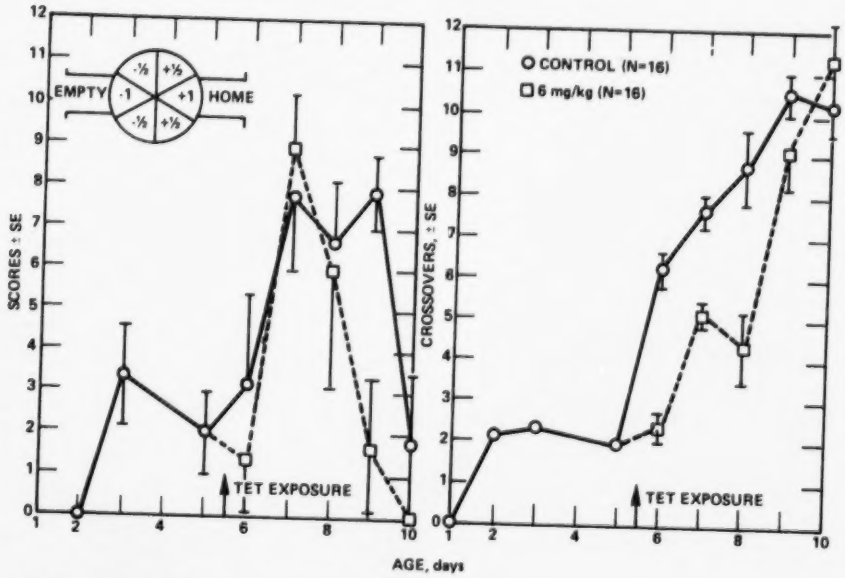

FIG. 7. Development of homing orientation and ambulation in control male rats and in those exposed to TET on postnatal Day 5 (N=16/group). No effect of TET on development of orientation was observed (left panel). Ambulation, measured as the number of crossovers in the 180-sec test period, was significantly depressed by TET (right panel). A diagrammatic representation of the test apparatus is shown in the upper left insert.

TABLE 2

EFFECTS OF TET EXPOSURE ON 5-MIN OPEN FIELD BEHAVIOR AND FIGURE-EIGHT MAZE ACTIVITY IN ADULT (110-117 DAY OLD) RATS. VALUES ARE EXPRESSED AS MEANS ±SE (N=9-10/DOSE)

	Males			Females		
	Control	3 mg/kg	6 mg/kg	Control	3 mg/kg	6 mg/kg
Latency (sec)	3.2 ± 0.7	4.2 ± 0.7	3.1 ± 0.9	4.3 ± 1.0	4.5 ± 1.0	5.5 ± 1.6
Crossovers	79 ± 9	68 ± 12	90 ± 8	99 ± 5	102 ± 14	111 ± 10
Rears	17 ± 2	13 ± 2	14 ± 4	24 ± 5	18 ± 2	17 ± 3
5 min Figure-Eight Maze	85 ± 10	79 ± 9	108 ± 9	100 ± 2	117 ± 9	110 ± 9

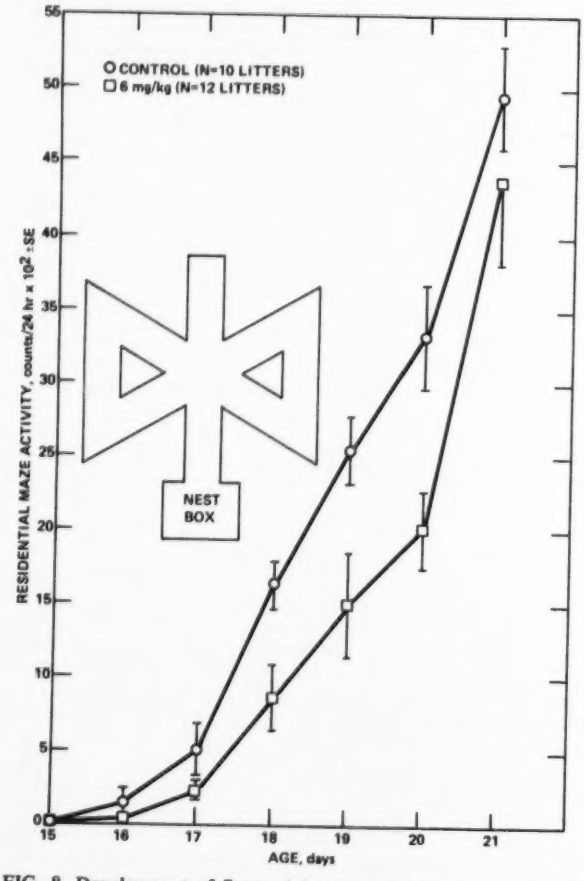

FIG. 8. Development of figure-eight maze activity in control and TET-dosed male rats (N=10–12 litters/treatment). Litters of 3 male pups were tested between 15–21 days of age. Mothers were restricted to the nest box. TET-exposure resulted in a transient delay in the development of motor activity.

recorded when an animal's position in the arena changed on succeeding observations. Therefore, a pup that moved to a different wedge every 10 sec for the entire 180 sec would receive a score of 18.

Nestbox study: A lactating female and three male littermates were placed in a nestbox (20×20×10 cm) containing food and water ad lib and nesting material. The nestbox was attached to the front alley of a figure-eight maze. A small hole (2×3 cm) allowed pups access to the maze but restricted the mother to the nestbox. In addition to the 4 control and 4 TET-dosed litters used in the homing orientation experiment, the data were pooled with two previous replications of the nestbox study (total N=10 control and 12 TET litters).

RESULTS

The mean orientation score of animals in both groups increased to a maximum at 7–9 days and declined on Day 10 (Fig. 7, left panel). These results do not agree with the findings of Altman and Sudarshan [2] who reported consistent homeward orientation by Day 10. In the present study, the decrease in orientation scores can be explained by the development of ambulation (Fig. 7, right panel). Since animals spend an increasing amount of time in locomotor activity they spend less time in homeward orientation. A steady increase in the number of crossovers is seen in the developing rat pup between 2 and 10 days of age. TET-dosed rats show a significant retardation in this development, $F(8,23)=5.09$, $p<0.001$.

The development of figure-eight maze activity is shown in Fig. 8. Some measurable motor activity occurs on Day 15, which corresponds to the age of eye opening, however little activity outside the nest box occurs at this age. Emergence clearly begins on Day 16; between 17 and 21 days of age there is a 10-fold increase in the activity of normal animals. A repeated measures ANOVA indicated that TET exposure caused a significant delay in this developmental pattern, $F(1,16)=12.95$, $p<0.005$. This effect of TET on locomotor activity is clearly seen between 18–20 days of age ($p<0.02$); however, by 21 days of age, control and experimental litters showed similar levels of activity.

DISCUSSION

These results demonstrate that TET produces both immediate and long lasting effects when administered to the developing rat. Following a single exposure on postnatal Day 5, TET produced a developmental delay in motor activity and a deficiency in rope descent. These early signs of neurotoxicity were associated with hyperactivity which persisted through 9 months of age.

In both experiments, the highest dose of TET caused considerable mortality; 12mg/kg produced 90% mortality vs 50% for the 9 mg/kg dose. These data suggest that the LD_{50} for TET in the 5-day-old rat pup is approximately 9 mg/kg. However, the exposure regimen employed in this study is somewhat unique in that the pups were randomly assigned to litters at birth and a within-litter dose-response was determined for both sexes. The possibility exists that with this design, the mortality curve would have an artifically steep slope owing to competition of pups within a litter. In this situation, the competition between pups for maternal care may also interact with treatment to exaggerate the mortality. This hypothesis is currently being tested in our laboratory. Nevertheless, the mortality observed in this study is in close agreement with that reported by Wender *et al.* [33] who found 50 percent mortality in 6-day old rat pups given 10 mg/kg TET.

Rat pups that received either 3 or 6 mg/kg TET showed a slight growth retardation during the preweaning period. Although growth retardation produced by undernutrition during the early postnatal period has been associated with delayed physical and behavioral development [15], the level of growth retardation is generally much greater than that observed in the present study. This is supported by the normal age of appearance of developmental landmarks including eye opening, startle response, and righting response in TET-treated pups. Other tests also revealed no differences in the development of TET-dosed pups (Table 1). Other neurotoxicants need to be tested, however, to determine if these tests can be indicators of perinatal toxicity.

In several preweaning tests, however, behaviors were disrupted by TET exposure. The ability of pups to descend a rope was impaired by TET. This deficit may indicate impairment in neuromotor development [2]. Simon and Volicer [27] found a delay in the forelimb grasp reflex in rats subjected to acute neonatal asphyxia. It is possible that similar forelimb impairment may be reflected in rope descent and in the grasp reflex.

TET-dosed pups also differed from controls in two preweaning activity measurements. When tested for homing orientation, TET-dosed pups were less active than controls even though the direction of orientation (i.e. home cage vs neutral cage) was not different. In addition, motor activity in the figure-eight maze showed a developmental lag; at a time when control pups began to move freely about the maze, TET-dosed pups were less active.

In addition to producing developmental delays, neonatal TET exposure resulted in hyperactivity which was present at 21 days of age and extended through the 9 month period of testing. This sequence of initial hypoactivity and later hyperactivity has also been reported following early exposure to other toxicants [6,35]. The parallel nature of the 23 hour activity, measured at 9 months of age, suggests that the rate of habituation to the test environment was not altered by TET exposure, but rather that overall activity levels were increased. This finding of hyperactivity following neonatal TET exposure has been replicated by Harry and Tilson [11] who used a similar exposure regimen. In addition to finding hyperactivity in neonatal TET-dosed rats, these authors found a decrease in the startle responsiveness and altered shuttle box avoidance performance.

There were no significant differences in open field behavior, either in animals tested during development or as adults. The test period used (5 min) may not be sufficient to detect treatment-related changes in behavior. Activity counts for the first 5 min in the figure-eight maze are consistent in showing no treatment effects (Table 2). Although activity in the 6 mg/kg treatment groups tended to be elevated in both test situations, these differences were not significant.

The profile of neurobehavioral toxicity following early postnatal exposure to TET differed from that previously seen following adult exposure. First, rats exposed to TET on postnatal Day 5 in this study were hyperactive as adults, whereas rats exposed as adults were hypoactive when tested in the same apparatus [24]. Second, the effect of neonatal exposure on activity was essentially irreversible whereas rats exposed as adults recover within 1 month after discontinuation of TET exposure [24]. Third, neonatal TET exposure permanently reduced brain weight whereas TET exposure in the adult produces an increase in brain weight, edema, and vacolation of myelin which is reversible following termination of exposure [17, 24, 28]. These data demonstrate that TET exposure produced long-term, irreversible damage to the developing nervous system but not to the mature nervous system.

Considerably more work is needed to establish whether a direct relationship exists between toxicant-induced hypoxia and developmental neurotoxicity. Although our results support this interpretation, no direct measurements of energy metabolism were made in this study. Nevertheless, the resultant hyperactivity produced by TET exposure is consistent with the behavioral change following neonatal exposure to carbon monoxide [7] and lead [20, 21, 26]. By disrupting energy metabolism and therefore producing cytotoxic hypoxia during early postnatal development, TET may interfere with one of the basic organizational processes in the developing nervous system. Bull *et al.* [4], for example, have demonstrated a relationship between the effects of perinatal lead-exposure on immature brain mitochondria and cytochrome accumulation. This, in turn, was associated with delays in synaptogenesis [18]. Therefore, the developmental neurotoxicity of these compounds may share a common mechanism which depends on their ability to interfere with energy metabolism during critical periods of development.

Finally, the present study has successfully utilized an acute postnatal exposure model to evaluate developmental neurotoxicity. Since the basic processes of neural development (i.e., cell division, migration and differentiation) are occurring during this time [32] in some brain areas, this exposure model should prove useful in evaluating the developmental neurotoxicity of neurotoxicants acting by a variety of mechanisms. This exposure regimen has several advantages: (1) it is less likely to be confounded by other teratogenic effects since other systems are more developed at birth, (2) it eliminates *in utero* effects, and (3) it is relatively easy to define and control exposure. Acute exposure on Day 5 was adequate to demonstrate the neurotoxicity of TET; the general utility of the model will be tested with other compounds.

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